

Case Report Rapport de cas

A cluster of trace-concentration methamphetamine identifications in racehorses associated with a methamphetamine-contaminated horse trailer: A report and analysis

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Abstract — Three low concentration methamphetamine “positive” tests were linked to use of a methamphetamine-contaminated trailer to transport the affected horses. This incident establishes methamphetamine as a human-use substance that can inadvertently enter the environment of racing horses, resulting in urinary methamphetamine “positives;” an interim regulatory cut-off of 15 ng/mL for methamphetamine in post-race urine is proposed.

Résumé — **Identifications de concentrations de méthamphétamine à l'état de traces chez des chevaux de course associées à une remorque contaminée : rapport et analyse.** Trois tests «positifs» de faibles concentrations de méthamphétamine ont été associés à l'utilisation d'une remorque contaminée par les méthamphétamines qui était utilisée pour transporter les chevaux affectés. Cet incident établit la méthamphétamine comme une substance à utilisation humaine qui peut pénétrer par inadvertance dans le milieu des chevaux de course, entraînant ainsi des tests d'urine «positifs»; un niveau intérimaire réglementaire de 15 ng/mL pour les méthamphétamines est proposé pour les tests d'urine après la course.

(Traduit par Isabelle Vallières)

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Introduction

Methamphetamine (*N*-methyl-1-phenylpropan-2-amine, C₁₀H₁₅N, molecular mass 149.2) is a phenylethylamine central nervous system stimulant and a Schedule I controlled substance in Canada. Methamphetamine exists as 2 enantiomers, *d*-methamphetamine and *l*-methamphetamine (Figure 1). *d*-methamphetamine is the more pharmacologically active enantiomer and is a DEA Schedule II controlled substance in the United States. In the US, *d*-methamphetamine is FDA approved for human use and is available as Desoxyn for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and other conditions. *l*-methamphetamine is available in the US in “over-the-counter” decongestant products such as Vicks VapoInhaler, in which the *l*-methamphetamine is identified as “levmetamfetamine.”

Racing laboratories now use Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) to test for methamphetamine. This has led to more than a thousand-fold increase in the

sensitivity of testing compared with traditional enzyme-linked immunosorbent assay (ELISA) testing and the identification of trace level concentrations of substances (such as methamphetamine) that would not previously have been identified. While these trace level identifications are pharmacologically insignificant, they may be reported as “positives” under “zero tolerance” regulatory guidelines required of racing chemists.

Methamphetamine is also illegally synthesized and may be widely available as a “street” drug of uncertain purity and enantiomeric (*d/l*) composition. The drug is produced in illicit synthesis laboratories that are kept undercover, and a large horse trailer would be well-suited to a mobile covert laboratory.

We now report an incident in which a recently purchased horse trailer contaminated with methamphetamine yielded a cluster of 3 trace-concentration post-race urinary methamphetamine identifications within a 48-hour period. The trainer's only other horse was transported in a different trailer and tested negative.

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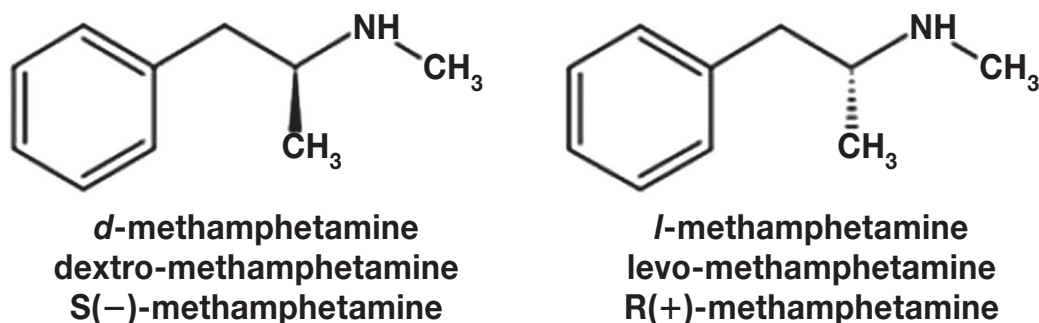


Figure 1. Methamphetamine, *N*-methyl-1-phenylpropan-2-amine exists as 2 enantiomers, *d*-methamphetamine and *l*-methamphetamine. *d*-Methamphetamine, the more active pharmaceutical, is a US DEA Schedule II controlled substance, while *l*-methamphetamine is available in over-the-counter preparations such as Vicks VapoInhaler.

The contaminated trailer was purchased by the horse trainer a few days prior to using it to ship horses on October 12th from Michigan to the 2014 Ajax Downs Quarter Horse Racing meet in Toronto. First contact of the affected horses with the methamphetamine-contaminated trailer was when they were loaded into the trailer for transportation to Toronto. The journey took 6 h, following which the horses were stabled in the Toronto area and transported to race at Ajax Downs on October 13th and 14th. The 3 horses that were transported in the newly purchased trailer raced successfully and were post-race tested, 2 on October 13 and 1 on October 14 (Table 1). Horse #1 won a maiden race on October 13th and tested positive for methamphetamine at 0.2 ng/mL. Horse #2 ran second on October 13th and tested positive at 0.056 ng/mL. Horse #3 won a starter allowance on October 14th and tested positive at 0.34 ng/mL. Horse #4 was transported from Michigan to Toronto in a different trailer. This horse raced and was tested on October 13th and did not test positive for methamphetamine or any other substance.

The samples were called methamphetamine “positive” in post-race urine tests performed by the official Canadian Laboratory, Maxxam Analytics of Burnaby, British Columbia. The referee split sample analyses were performed by Industrial Laboratories, Denver, Colorado, which confirmed the identifications and quantified the amounts present. The analytical methodologies available at that time in either laboratory did not distinguish between the *d*- and *l*-enantiomers of methamphetamine; however, more recent work at Industrial Laboratories has confirmed that the methamphetamine was present predominantly as the *d* form. No information is available concerning the presence of metabolites of methamphetamine — principally amphetamine — presumably because such information was not required and/or requested.

Laboratory testing for methamphetamine

In the United States, federal workplace testing identification of 80% or more *l*-methamphetamine suggests over-the-counter drug use, *versus* lower levels, which suggests illicit drug use (1,2). Also, the United States federal workplace testing protocol of the Substance Abuse and Mental Health Services Administration (SAMHSA) requires that methamphetamine/amphetamine be present in urine at > 500 ng/mL by immunological testing and

that methamphetamine be confirmed by mass spectrometry at > 250 ng/mL, followed by confirmation of the metabolite, amphetamine, at a concentration > 100 ng/mL before a positive methamphetamine report may be issued (3).

This compares to the cut-offs suggested in “The Canadian Model for Providing a Safe Workplace: Drug and Alcohol Guidelines and Work Rule v5.0” of 500 ng/mL for total amphetamines (including *d*-/*l*-methamphetamine), with verification via GC-MS having a cut-off of 250 ng/mL for specific compounds (4). While these suggested levels are cutoffs recommended by a professional organization of construction workers, their levels seem to match SAMHSA, and indicate similar levels are likely in Canada.

Scientific literature yields numerous reports showing that methamphetamine persists in the environment and in wastewater (5,6). These findings speak to inconsequential levels associated with of both licit and illicit human use in the environment, and also demonstrate its ability to persist in the environment (6,7), consistent with the presence in the contaminated trailer in the current matter.

The close spatial and temporal clustering of these 3 trace-concentration, methamphetamine identifications is an extremely unusual event, both in the long time professional experience of the involved trainer, and in the scope of the human and equine drug testing experience of the authors. In particular, the trainer in question in this matter was in good standing with the Ontario Racing Commission (ORC). Furthermore, it is also extremely unusual to see 3 very low/trace concentration ARCI class 1 substance identifications occurring in a small group of horses within a 48-hour period.

The ORC personnel investigating this matter also recognized the unusual nature of this cluster of methamphetamine identifications. Following a series of interviews with the trainer and her staff, and considering the overall circumstances of this matter, the ORC investigators elected to take a number of samples from the trailer in which the horses in question were transported from Michigan to Toronto for methamphetamine testing, with a particular focus on the manger areas of the trailer.

Analysis of these trailer samples showed that at least 1 of these samples taken from the manger area of the trailer tested positive for methamphetamine at a concentration of 22 ng/g, or 22 parts per billion. This positive finding for methamphetamine

Table 1. Transportation, racing and urinary methamphetamine analytical data on 4 horses transported to Ajax Downs, Toronto, Ontario, for racing on October 13 and 14, 2014

Horse	Race date (2014)	Race type	Race result	Trailer	Time in trailer A (hours)	Sample (Maxxam lab test result)	Split sample (Industrial lab test result)
Fames Winner	Oct 13	Maiden	Win	A	6+	Positive	0.20 ng/mL
Chasing Royalty	Oct 13	Allowance	Place	A	6+	Positive	0.056 ng/mL
JJ Maxwell	Oct 14	Starter Allowance	Win	A	6+	Positive	0.34 ng/mL
Jess What To Wear	Oct 13	Allowance — non winner of two	Win	B	0	Negative	N/A

The Maxxam laboratory and Industrial laboratories tested split samples. N/A — Not available.

in the trailer is entirely consistent with the underlying cause of this cluster of trace urinary concentration methamphetamine identifications being this trailer's contamination by methamphetamine, since the horses spent 6 h in the contaminated trailer during their journey from Michigan to Toronto while being shipped to Ajax Downs.

These interpretations were also recognized by the lead ORC investigator, who testified at the Stewards hearing that the methamphetamine identifications were apparently the result of inadvertent exposure of the horses to the contaminated trailer and that the identifications did not in any way reflect on the trainer involved.

The scientific literature on methamphetamine in the horse supports the ORC investigators analysis and interpretations. Work by Ray et al (8) shows that after IM administration of 150 mg methamphetamine to horses the urinary concentrations of methamphetamine peak at a mean of about 7400 ng/mL (range: 3240 to 17 930 ng/mL) at 4 h post-administration and thereafter decline. The urinary concentrations of methamphetamine reported in the current matter are between 20 000 and 130 000 times lower (0.34 and 0.056 ng/mL samples, respectively) than the mean peak urinary concentration reported by Ray et al (8) after their methamphetamine administrations.

A second conclusion that may be drawn from the trace concentrations of methamphetamine observed in these samples is that the amounts of methamphetamine present in the urine samples of these animals are 10^5 of the concentration which would be consistent with production of a pharmacological effect (8). As such, it is a reasonable conclusion that there was no effect on the racing performance of the horses in question.

The identification of trace level concentrations of human-use substances in the post-race urine samples of racing horses is a not an uncommon occurrence. Beginning in the 1990s, the ongoing increases in the sensitivity of drug and medication testing in racehorses has created a need to develop regulatory thresholds/cut-offs for Endogenous, Dietary and Environmental substances (EDEs) in post-race urine samples (2,9).

Establishing regulatory cut-offs (thresholds)

The first trace environmental substance to come to the attention of racing regulators was caffeine, and Florida racing regulators introduced a plasma regulatory threshold of 100 ng/mL, a cut-off since adopted by the Association of Racing Commissioners International (ARCI) and now in place in at

least 10 US jurisdictions. In Florida, this threshold has since been adjusted to 200 ng/mL in urine (10).

Another environmental substance regulatory cut-off used in several states is for benzoylecgonine (BZE), the major urinary metabolite of cocaine, which is efficiently excreted at relatively high concentrations in equine urine. Like caffeine, cocaine is widespread in the human environment and is found in measurable quantities on about 80% of paper money in the US. Though exposure to the quantity of cocaine on paper money is generally insufficient to produce a pharmacological effect, ingestion by a horse of an amount of cocaine not uncommonly found on a single dollar bill in general circulation can produce a measurable concentration of BZE in the horse's urine — a potential positive test (11).

One outcome of the widespread environmental presence of trace amounts of cocaine is that US Department of Health and Human Services human workplace drug testing has a BZE urinary screening "cut-off" of 150 ng/mL and a confirmation cut-off of 100 ng/mL (12). This threshold was essentially adopted by a number of racing commissions, and equine urinary BZE thresholds are in place in at least 7 US racing jurisdictions ranging from 50 to 150 ng/mL (2,11).

Morphine is also an ARCI class 1 substance that is occasionally identified in post-race equine urine samples, usually as classic clusters associated with inadvertent feed contamination, although isolated cases occur in which the source of the morphine remains unidentified. Again, the solution to this problem has been the introduction of equine urinary cut-offs varying from 50 to 120 ng/mL depending on the jurisdiction involved (2,12,13). These equine urinary cut-offs are considerably more rigorous than the 2000 ng/mL opiate cut-off used in US Department of Health and Human Services human workplace drug testing (3).

Consistent with the above referenced well-established regulatory cut-offs for environmental substances, it is now apparent that the increased sensitivity of testing for methamphetamine has created a need for its own regulatory cut-off in equine testing. In the absence of a defined regulatory cut-off, "positives" are reported down to the limit of detection of the analytical method. The sensitivity of testing for methamphetamine in this current matter, at a limit of detection of about 0.05 ng/mL (50 pg/mL), is 1000 times less concentrated than the cut-offs for caffeine, benzoylecgonine, and morphine in post-race urine samples, and 10 000 times less than the 500 ng/mL amphetamine screening human cut-off of the US Department of Health

and Human Services (3). The only question that remains is to determine precisely what the appropriate concentration for this urinary methamphetamine cut-off should be. Review of data generated by Ray et al (8), which showed a mean peak urinary concentration of methamphetamine of 7400 ng/mL following administration of a 150 mg dose of methamphetamine, suggests that a 1000-fold lower urinary methamphetamine concentration, as seen in the cluster of horses in question, is unlikely to be associated with a pharmacological effect. Therefore, a urinary methamphetamine concentration of < 15 ng/mL in urine would be unlikely to be associated with any possible effect on a horse at the time of racing, and as such would be an appropriate interim environmental cut-off for methamphetamine identifications in post-race urines.

With respect to developing an environmental substance threshold or cut-off for methamphetamine in post-race urine samples, it may be instructive to consider the protocol used in US Department of Health and Human Services human workplace drug testing for methamphetamine. To report a urine sample positive for methamphetamine the sample must first yield an initial immunoassay result of more than 500 ng/mL for combined amphetamine/methamphetamine reactivity, then the laboratory must confirm by Mass Spectrometry that the sample contains more than 250 ng/mL of methamphetamine and also that the sample contains at least 100 ng/mL of the metabolite, amphetamine. This is to show that the methamphetamine has actually passed through the individual in question. This precaution is required as early GC/MS confirmation procedures used in human drug testing were shown to convert ephedrine to methamphetamine and it was necessary to rule out analytical neogenesis of methamphetamine from ephedrine.

It is well-established that individuals living in or exposed to clandestine methamphetamine laboratories become contaminated with methamphetamine and will test positive for methamphetamine at much higher concentrations than is required for human workplace positive identifications. These positives occur not because they are actively using the drug, but simply from environmental exposure; this is best seen with regard to toxicology studies on children and infants found living in places where methamphetamine is being manufactured, according to a Department of Justice review (14). With regard to the difficulty of removing methamphetamine residues from such an environment, this report notes that *"Normal cleaning will not remove methamphetamine and some of the chemicals used to produce it. They may remain on eating and cooking utensils, floors, countertops, and absorbent materials. Toxic by-products of meth manufacturing are often improperly disposed outdoors, endangering children and others who live, eat, play, or walk at or near the site"* (14). One author (TFS) has recommended that first-responders and police officers be screened both before and after their exposure to drug laboratories or drug storage facilities.

Methamphetamine, a substance of human use/abuse, can inadvertently become part of the environment of racing horses, yielding trace urinary methamphetamine "positives" in post-race testing. As such, there is a need to establish a cut-off or threshold for methamphetamine in equine drug testing, as has been done for other environmental substances (caffeine, BZE,

morphine, etc.) to avoid unjustly penalizing the connections of the horse and tarnishing the image of racing. We therefore suggest an interim urinary threshold/cut-off for methamphetamine of at least 15 ng/mL, similar to thresholds already established for other environmental substances, and well below the 500 ng/mL cut-off used by the US Department of Health and Human Services in human workplace drug testing. This interim threshold would be appropriate *prima facie* until a review leads to a formal regulatory decision to establish a threshold that takes into account metabolite concentrations and effects of other variables, such as urine pH, on racehorse sample concentrations. While it may need to be raised or lowered in the future, 15 ng/mL is at this time a conservative estimate based upon the current findings in the case presented.

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Note:

In Ontario, these matters went to a Steward's hearing on October 23rd, 2014; despite the weight of evidence for trainer innocence, the trainer was fined \$5000 and suspended for 1 year. The matter was appealed to the Ontario Racing Commission (ORC), which held hearings related to this matter in July to September 2015. On February 8th, 2016 the ORC allowed the appeals and set aside the Steward's penalties. The ORC noted the substantial increases in sensitivity of equine drug testing, consistent with the very low levels of methamphetamine identified in these horse urines, levels in the opinion of the ORC with no possible impact on the performance, health and safety of horses, and levels consistent with inadvertent environmental contamination. The ORC also noted the need *"to set limits high enough to cut-off the environmental noise and low enough to stop performance enhancement."* In this communication we have presented our best estimate of this as 15 ng/mL interim regulatory cut-off for methamphetamine in post-race horse urine.

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